



AssayMax Human Tissue Factor (TF) ELISA Kit

Catalog No. ET1002-1

Introduction

The transmembrane protein Tissue factor (TF) is the physiologic trigger of coagulation in normal hemostasis. TF binds and allosterically activates factor VII. The TF-VIIa complex cleaves factor IX and X, leading to thrombin generation (1). Inducible expression of TF in a variety of pathological conditions, including gram-negative sepsis and acute coronary syndromes, is associated with life-threatening thrombosis (2, 3). In sepsis, TF expression within the vasculature leads to disseminated intravascular coagulation (4). TF also plays important roles in vasculogenesis, metastasis, and tumor-associated angiogenesis (5, 6, 7).

Principal of the Assay

The AssayMax Human Tissue Factor (TF) ELISA kit is designed for detection of human TF in plasma, serum, saliva, tissue, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TF in less than 5 hours. A polyclonal antibody specific for TF has been pre-coated onto a 96-well microplate. TF in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for TF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **TF Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against TF
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **TF Standard:** Human TF in a buffered protein base (1600 pg, lyophilized).

- **Biotinylated TF Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against TF (80 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard at 2-8⁰C before reconstituting with Diluent and at -20⁰C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel pipettes)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:2 with MIX Diluent and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:2 with MIX Diluent and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Lysates:** The cultured cells are lysed and solubilized with 15 mM octyl-β-D-glucopyranoside at 37⁰C for 15 minutes. Collect fresh cell lysates and assay. The undiluted samples can be stored at -20⁰C or below.
- **Tissue:** Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000x g for 20 min. Collect the supernatant, measure the protein concentration and assay. The undiluted samples can be stored at -20⁰C or below.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.

- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **TF Standard:** Reconstitute the 1600 pg of TF Standard with 2 ml of MIX Diluent to generate 800 pg/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution 1:2 with equal volume of MIX Diluent to produce 400, 200, 100, 50, and 25 pg/ml of solutions. MIX Diluent serves as the zero standard (0 pg/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[TF] (pg/ml)
P1	1 part TF Standard (800 pg/ml)	800.00
P2	1 part P1 + 1 part MIX Diluent	400.00
P3	1 part P2 + 1 part MIX Diluent	200.00
P4	1 part P3 + 1 part MIX Diluent	100.00
P5	1 part P4 + 1 part MIX Diluent	50.00
P6	1 part P5 + 1 part MIX Diluent	25.00
P7	MIX Diluent	0.00

- **Biotinylated TF Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20⁰C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20⁰C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30⁰C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated TF Antibody to each well and incubate for two hours.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.

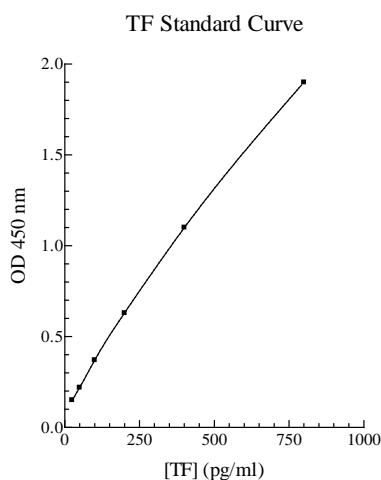
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of TF is typically ~ 20 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.2% and 7.2% respectively.
- This assay recognizes both natural and recombinant human TF apoprotein and TF/FVII complexes.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
No Dilution	96%	96%
1:2	101%	100%
1:4	111%	110%

Recovery

Standard Added Value	30 – 300 pg/ml
Recovery %	80-115 %
Average Recovery %	97.5 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	80% (Suggest No Dilution for plasma)
Monkey	10%
Mouse	100% (Suggest No Dilution for plasma)
Rat	20%
Swine	80% (Suggest No Dilution for plasma)
Rabbit	100% (Suggest No Dilution for plasma)
Bovine	1%

References

- (1) Ruf, W. and Edgington, T.S. (1994) *FASEB J.* 8:385
- (2) Fuster, V. *et al.* (1996) *Haemostasis* 26:269
- (3) Leatham, E. *et al.* (1995) *Br. Heart. J.* 73:10
- (4) Drake, T.A. *et al.* (1993) *Am. J. Pathol.* 142:1
- (5) Carmeliet, P. *et al.* (1996) *Nature* 383:73
- (6) Ruf, W. and Mueller, B.M. (1996) *Curr. Opin. Hematol.* 3:379
- (7) Zhang, Y. *et al.* (1994) *J. Clin. Invest.* 94:1320

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